Original Article

Journal of Apiculture 30(3) : 155~160 (2015)

Nutritional Evaluation of Four Commercially Available Pollen Patties in Korea

Sampat Ghosh and Chuleui Jung*

Department of Bioresources Sciences, Andong National University, Republic of Korea

(Received 4 September 2015; Revised 24 September 2015; Accepted 26 September 2015)

Abstract

Pollen patty is one of the requirements for commercial beekeeping especially in early spring when overwintered honey bee colony begin production of brood. The quality and quantity of pollen patty substituting the natural sources of protein and carbohydrate could determine the spring colony production, but limited information is available in nutritional contents of commercial pollen patties in Korea. We analysed nutrient contents of four commercially available pollen patties and compared with recommended requirement of essential amino acids for honey bee. The results revealed that protein content of different pollen patties ranged 18.9 to 22.4%.The recovery of the protein in terms of amino acids was found more than 90%. The analytical data showed that phenylalanine was limiting while sufficient tyrosine was present. High tyrosine levels could decrease the need for dietary phenylalanine. Arginine was found another limiting amino acid and histidine was present in marginal level. Fat content of four patties ranged 0.7 to 1.3%. Overall, this study results showed that protein enforced pollen patty could serve as natural pollen alternative in early spring.

Key words: Protein, Spring build-up, Substitute, Essential amino acid

INTRODUCTION

Food is essential for any living organism to sustain life on the earth and honey bee is not an exception. Pollen is the major source of honey bee diet providing a wide range of essential nutrients including protein, carbohydrates, lipids, minerals and vitamins (Human and Nicolson, 2006). The quality and quantity of pollen collected by honey bee affect brood rearing, development, longevity and productivity of colony. During early spring lengthening day time and new source of pollen and nectar stimulate brood rearing and later in the spring gradually the population of the colony expands rapidly and proportion of young bees increases. The period is considered as high honey flow season. In the fall a reduction in the amount of pollen and nectar causes reduced brood rearing and diminishing the population. Coming to existence on the earth 40 million year ago honey bee adapts well to battle with the condition of dearth season when there is insufficient forage to feed the colony (Burleigh and Whalley, 1983). Collection of greater amount of nectar and pollen in time of abundance and make store of honey and pollen to use in winter, dragging drones out of the hives are among the strategies they follow to manage the colony in time of food scarcity. It is often a common practice for the bee keepers to augment the food storage in winter by supplying artificially synthesized food known as 'pollen patty'. In general 'pollen patty' is of two types namely pollen supplement patty containing pollen and pollen substitute patty containing no pollen.

The patty, pollen substitute flattened cake about 0.5 inches thick usually is placed on the top bars directly over the

*Corresponding author. E-mail: cjung@andong.ac.kr

centre of the cluster. The top of the cake must be covered with waxed paper to prevent dehydration and hardening of the patty. The inner cover, when used should be inverted with the rim side down to provide space to the cake. The new patty should be added before the old one is consumed. In summer season of 5 months duration a colony consisted of 30000 bees and rearing 150000 bees consumes about 20kg of pollen and 60kg of honey (Seeley, 1985). As a rule of thumb 1kg of pollen is required for every 1kg (about 9000 to 10000 bees) bees and the same rule generally is followed in providing the pollen substitute to the bees in winter season. However, the quantity of the feed is not only important but also important is the quality of feed for overwintering colony. Honey bee colony as a super-organism comprises three levels of nutrition viz. colony nutrition, adult nutrition and larval nutrition (Brodschneider and Crailsheim, 2010). Moreover physiological and nutritional needs of workers, drones and queens also differ (Hrassnigg and Crailsheim, 2005). Though there are few significant scientific works demonstrates the requirement of protein ensuring bee health and strong population but our understanding the role of essential lipids, minerals and vitamins for brood rearing and development of adults is still limited. Thus, there is no such formulation for the synthesis of pollen patty or feed supplement for bee colony in Korea. Therefore, the study was undertaken to assess the nutritional quality of the pollen patties commercially available in the market.

MATERIALS AND METHODS

Sample collection and preparation

Samples of different pollen patties were obtained from the retailed sources of four different companies of Korea namely from Sample 1 to 4. The samples were taken to laboratory, then oven-dried (50°C), ground to a powder and prepared as a dry matter (DM) for further analyses. All solvents and chemicals used in the study were of analytical grade.

Proximate analyses

Proximate compositions moisture content, crude protein, crude fat, crude ash, crude fibre, and nitrogen free extract (NFE) were analyzed according to the standard methods recommended by Association of Official Analytical Chemists (AOAC, 1990).

Moisture percentage was calculated by drying the sample in an oven at 100°C for 2 h. The dried sample was put into desiccators and allowed to cool and reweighed. The process was repeated until constant weight was obtained. Crude protein (%N×6.25) was determined by the Kjeldahl method (AOAC, 1990). Crude fat was analysed using a Soxhlet extractor (AOAC, 1990).

Crude ash was measured by dry combustion method using a muffle furnace. Crude fibre was determined through double digestions, first with sulphuric acid and then with sodium hydroxide. Except moisture all the analyses was performed on the dry matter. The percentage of nitrogen free extract (NFE) supposedly representing soluble carbohydrate was determined by subtracting all of the components (crude protein, crude lipid, crude fibre and ash) from 100.

All the analyses were carried out in triplicate and the results were expressed as mean±standard deviation.

Amino acid analysis

Amino acid composition was determined by an Amino Acid analyzer S433 (Sykam GmbH, Germany) following the standard method of AOAC (1990). The ground samples were hydrolyzed in 6 N HCl for 24 h at 110°C under nitrogen atmosphere and then concentrated with rota evaporator. The concentrated samples were reconstituted with sample dilution buffer supplied by the manufacturer (0.12N, pH 2.20). The hydrolyzed samples were analyzed for amino acid composition. The operating condition of the amino acid analyzer was as the following:

Instrument: Amino acid analyzer (Sykam GmbH, Germany)
Column: LCA K07/Li (PEEK-column 4.6×150mm)
Application: Physiological
Detector: Photometer (570nm, 440nm)
Detection principle: Ninhydrin reaction
Inert gas: N_2

Fatty acid analysis

Fatty acid composition was analyzed by gas chromatographyflame ionization detection (GC-14B, Shimadzu, Tokyo, Japan) following the standard method of Korean Food Standard Codex (2010). The samples were derivatised fatty methyl esters (FAMEs) according to the method of Lepage and Roy (1986). Identification and quantification of FAMEs were accomplished by comparing the retention times of peaks with those of pure standards purchased from Sigma-Aldrich Co. and analyzed under the same conditions. The results were expressed as the percent ratios of individual fatty acids in the lipid fraction. The operating condition of GC for fatty acid analysis was as the following:

Instrument: Gas Chromatography
(GC-14B, Shimazdu)
Column: SP-2560
Detector: Flame Ionization detector (FID)
Carrier gas: N_2, 300 KPa
Initial temperature: 170°C
Initial time: 0 min
Program rate: 1°C /min
Final temperature: 205°C
Final time: 5 min
Split ratio: 100:1

Minerals analysis

Minerals were analysed following the standard method of Korean Food Standard Codex (2010). The dried, powder samples was digested with nitric and hydrochloric acid (1:3) at 200°C for 30 min. Each sample was filtered using Whatman filter paper (0.45μm) and stored into washed glass vials before analysis. Minerals were analysed by inductively-coupled plasma-atomic emission spectrophotometer (ICP-AES; Intrepid II XDL, Thermo Fisher Scientific, Bath, UK).

RESULTS AND DISCUSSION

A summary of nutritional content of four pollen patties is presented in Table 1. The moisture contents of these patties were reported within the range of 10 to 13% which is similar to most of the reported value of studied pollens (Roulston and Cane, 2000). The less moisture content also suggests less susceptibility to microbial contamination. Soluble carbohydrate was represented as nitrogen free extract (NFE). NFE content of these pollen patties ranged between 67 to 69.3%. Lack of carbohydrate limits the larvae reared in spring especially in time of scarcity of nectar and pollen source and in winter in time of depletion of stored food or after harvesting honey without adequate replacement of carbohydrates (Brodschneider and Crailsheim, 2010).

Protein always receives primary attention and is considered a reliable measure of the nutritional value of a food or feed. The results revealed that protein content of different pollen patties ranged 18.9 to 22.4%. With the value 22% sample 2 contained the highest amount of protein among four. Protein has been found a major factor to limit rapid increase of colony population. Pollen is the best source of protein for honeybee nutritional requirement and the protein content can vary from 2.5 to 61% according to the floral source (Roulston *et al*., 2000). Research revealed that the pollen of animal-pollinated plant is not richer in protein than that wind-pollinated plants and it supports that honeybee collects pollen not from one source but from different sources to satisfy their nutritional requirement. However pollen with less than 20% crude protein could not satisfy a colony's requirements for optimum production. Thus there is a scope to fortify these patties with protein. However the quality of protein as related to nutrition depends upon the amino acid content of the protein. Thus the amino acid composition of the patties have been analysed and presented in Table 2. The recovery of the protein in terms of amino acids was found more than 90%. At least 17 amino acids of proteinergic importance were determined including 10 essential amino acids for honey bee. Methionine and tryptophan were determined partially, presumably due to the limitation of acid hydrolysis process. Lysine was found predominant essential amino acid followed by leucine. The proportion of non-essential amino acids was found higher than the proportion of essential amino acids in all the cases. The analytical data suggested that phenylalanine was found limiting amino acid while comparing to the minimum requirement suggested by DeGroot (1953). But at the same time these

Table 1. Proximate composition (mean±SD) of different pollen patties

Moisture	Moisture	Protein	Fat	Fibre	Ash	NFE
	as-is based	Dry matter based (%)				
Sample 1	13.2±0.92	18.9±0.28	1.1±0.24	4.3±0.20	6.4±0.05	69.3±0.42
Sample 2	11.5±1.48	22.4±0.83	0.7±0.03	2.9±0.38	5.9±0.05	68.1±0.48
Sample 3	10.2±0.15	19.2±0.47	1.1±0.13	6.6±0.92	6.0±0.23	67.1±0.91
Sample 4	11.3±1.59	19.3±0.85	1.3±0.21	3.9±1.36	6.2±0.44	69.4±1.13

Table 2. Amino acid composition of different pollen patties and comparison with the minimum levels of requirement

Amino acid	Sample 1*		Sample 2		Sample 4		Minimum level required **
	g/100g DM	% of total amino acids	g/100g DM	% of total amino acids	g/100g DM	% of total amino acids	g/100 g protein
Valine***	0.8	4.2	1.1	5.3	0.9	4.9	4.00
Isoleucine*	0.8	4.2	1.0	5.2	0.4	2.3	4.00
Leucine*	1.3	6.8	1.7	8.2	2.3	12.4	4.50
Lysine*	4.7	24.7	3.2	15.8	2.9	15.3	3.00
Threonine*	1.2	6.4	0.5	2.7	1.9	10.1	3.00
Phenylalanine*	0.03	0.2	0.03	0.1	0.04	0.2	1.50
Histidine*	0.3	1.4	0.3	1.4	0.3	1.8	1.50
Arginine*	0.3	1.6	0.2	1.0	0.6	3.0	3.00
Methionine*	0.001	0.007	0.005	0.026	0.007	0.038	1.50
Tryptophan*	ND	--	ND	--	0.02	0.09	1.00
Tyrosine	0.8	4.4	1.2	5.7	1.0	5.5	
Aspartic acid	1.1	5.7	3.1	15.4	1.3	6.9	
Serine	1.1	6.1	1.1	5.7	1.5	7.9	
Glutamic acid	3.7	19.6	3.6	17.8	3.3	17.6	
Glycine	0.8	4.1	1.0	4.8	0.9	4.7	
Alanine	0.9	4.5	1.1	5.6	1.0	5.2	
Cysteine	0.2	0.8	0.2	1.0	0.1	0.8	
Others	1.0	--	0.8	--	0.4	--	

*Data of Sample 3 are not analyzed for amino acid.

**Minimum requirement was adopted from DeGroot, 1953.

***Bold characters indicates essential amino acid.

patties were found good source of tyrosine. This is important because high tyrosine levels could decrease the need for dietary phenylalanine. Although in the study methionine was determined in low level, but higher level of cysteine was determined. Methionine, cysteine, homocysteine and taurine are four common sulphur containing amino acids but only first two are incorporated into protein i.e. proteinergic. Higher level of cysteine thus could be expected to decrease the dietary requirement of methionine. Arginine was found another limiting amino acid and histidine was present in marginal level. The distribution of essential amino acids was found heterogeneous. Proportion of lysine, leucine was very high in all the pollen patties. In contrast threonine in sample 2 and isoleucine in sample 4 were found comparatively low thus, limiting. Therefore there is a scope to fortify these pollen patties with limiting amino acids. Among non essential amino acids Glutamic acid was found predominant.

Fat content of four patties ranged 0.7 to 1.3%. Literature suggests that fat content of pollen ranged between 0.8 to 31.7% (Farag *et al*., 1978; Evans *et al*., 1987; Roulston and Cane, 2000). Fat including fatty acids and sterols are

Table 3. Fatty acid composition of different pollen patties

Fatty acids	Sample 1		Sample 2		Sample 3		Sample 4	
	mg/100g DM	% of total FA	mg/100g DM	% of total FA	mg/100g DM	% of total FA	mg/100g DM	% of total FA
Caproic acid C6:0	0	0	0.2	0.1	0	0	0.2	0.1
Caprylic acid C8:0	0	0	0.7	0.2	0.3	0.1	0.3	0.2
Capric acid C10:0	3.7	1.0	6.3	2.2	4.1	1.7	2.8	1.4
Lauric acid C12:0	2.4	0.6	2.1	0.7	2.5	1.0	1.2	0.6
Myristic acid C14:0	7.2	1.9	5.4	1.9	5.4	2.2	4.8	2.5
Pentadecanoic acid C15:0	0	0	0.4	0.1	0	0	0.3	0.2
Palmitic acid C16:0	114.6	29.9	94.2	32.6	67.4	28.0	58.5	29.9
Margaric acid C17:0	0	0	0.7	0.2	0.7	0.3	0.6	0.3
Stearic acid C18:0	14.6	3.8	11.5	4.0	7.7	3.2	7.1	3.6
*SFA Sub total	142.5	37.1	121.5	42.0	88.1	36.6	75.8	38.8
Tetradecenoic acid C14:1	0	0	0.4	0.1	0	0	0	0
Hexadecenoic acid C16:1	11.4	3.0	14.6	5.0	4.9	2.0	4.6	2.4
Oleic acid C18:1	31.8	8.3	19.6	6.8	13.1	5.4	12.2	6.2
Eicosenoic acid C20:1	121.5	31.7	92.1	31.8	96.8	40.2	75.8	38.8
Nervonic acid C24:1	3.1	0.8	2.4	0.8	2.3	1.0	2.4	1.2
*MUFA Sub total	167.8	43.7	129.1	44.6	117.1	48.7	95	48.6
Linoleic acid C18:2 n-6	73.3	19.1	37.5	13.0	35.4	14.7	24.3	12.4
EPA C20:5 n-3	0	0	1.2	0.4	0	0	0.4	0.2
*PUFA Sub total	73.3	19.1	38.7	13.4	35.4	14.7	24.7	12.6
SFA/USFA	--	0.6	--	0.7	--	0.6	--	0.6

*SFA denotes saturated fatty acid, MUFA does monounsaturated fatty acid, PUFA does polyunsaturated fatty acid and USFA represents total unsaturated fatty acids.

important source of energy used for the synthesis of reserve fat and glycogen and contribute to the production of royal jelly (Singh *et al*., 1999). Following the variation of fat content patties also vary in the relative proportion of fatty acid content and diversity. 16 fatty acids of 3 different categories saturated, monounsaturated and polyunsaturated were determined. Unsaturated fatty acid fraction (58 to 62.9%) was found higher than that of saturated fatty acid (36.6 to 42%) in all four patties. Palmitic acid was found predominant followed by stearic acid among saturated fatty acids. Eicosenoic acid was predominate followed by oleic acid among monounsaturated fatty acids and this observation was in agreement to the report on bee collected and bee stored *Aloe gretheadii* pollen (Human and Nicolson, 2006). The major fraction of pollen fat generally consists of palmitic, oleic, linoleic and linolenic (Manning, 2001). Certain fatty acids like linoleic, linolenic, myristic acid possess bactericidal properties thus important for colony hygiene. On the other hand pollens high in oleic acid and palmitic acid shows greater role in honey bee nutrition (Manning, 2001). Thus the patties could be better source for fat for the bee nutrition.

The mineral i.e. sodium, potassium, magnesium and calcium contents are reported in Table 4. The most abundant mineral was found calcium and sodium content

Table 4. Mineral composition of commercially available different pollen patties (mg/100g dry matter)

	Sample 1	Sample 2	Sample 3	Sample 4
Sodium	57.4	32.6	40.0	30.0
Potassium	84.4	116.2	70.9	83.8
Magnesium	94.2	113.2	95.8	96.9
Calcium	566.5	569.6	566.9	562.9

was found very less. Minerals requirement of honey bee is poorly understood. High amount of potassium, phosphate and magnesium is required for the sustenance for insects and thus presumably is important for honey bee. Excessive sodium and calcium level show to be toxic for honey bees. In general bee pollen was always found to contain higher amount of potassium and lower amount of sodium. Potassium content of these patties was found lesser than that reported for 154 pollen samples from Brazil, and 12 pollen samples from China (Morgano *et al*., 2012; Yang *et al*., 2013).Opposite trend was followed for sodium and calcium content and magnesium content was found within the range reported in other studies (Yang *et al*., 2013; Morgano *et al*., 2012; Szczesna, 2007; Carpes *et al*., 2009).

Perhaps the mostly argued and discussed subject in beekeeping management is the methods of overwintering and spring population build-up. In the temperate region like in Korea, high population reduction during cold winter could be rapidly compensated and colony can become strong by provisioning adequate amount and quality of pollen substitute. The findings of the study suggests that with fortification of certain protein and limiting amino acids these pollen patties could be a valuable source of nutritional supplement in time of insufficient foraging of pollen and nectar.

ACKNOWLEDGEMENTS

SG is supported from Andong National University BK21 Plus program from Ministry of Education. This research was partially supported by Bio industry Technology Development Program (Nr. 312027-3), Ministry of Agriculture, Food and Rural Affairs.

LITERATURE CITED

AOAC. 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington DC, USA.

Brodschneider, R. and K. Crailsheim. 2010. Nutrition and health in honey bees. Apidologie. 41: 278-294.

Burleigh, R., P. Whalley. 1983. On the relative geological age of amber and copal. J. Nat. Hist. 17: 919-921.

Carpes, S.T., G.B. Mourao, S.M. de Alencar, M.L. Masson. 2009. Chemical composition and free radical scavenging activity of *Apis mellifera* bee pollen from Southern Brazil. Braz. J. Food Technol. 12(3): 220-229.

DeGroot, A.P. 1953. Protein and amino acid requirements of the honeybee (*Apis mellifera* L.). Physiol. Comp. Ocol. 3: 1-83.

Evans, D.E., N.E. Rothnie, M.V. Palmer, D.G. Burke, J.P. Sang, R.B. Knox, E.G. Williams, E.P. Hilliard and P.A. Salisbury. 1987.Comparative analysis of fatty acids in pollen and seed of rapeseed. Phytochemistry. 26: 1895-1897.

Farag R.S., A.M. Youseff, M.A. Ewiss, S.A.S. Hallabo. 1978. Long chain fatty acids of six pollens collected by honeybees in Egypt. J. Apicult. Res. 17: 100-104.

Hrassnigg, N., K. Crailsheim. 2005. Differences in drone and worker physiology in honeybees (*Apis mellifera* L.). Apidologie. 36: 255-277.

Human, H., S.W. Nicolson. 2006. Nutritional content of fresh, bee-collected and stored pollen of *Aloe greatheadii* var. *davyana* (Asphodelaceae). Phytochemistry. 67: 1486-1492.

Korean Food Standard Codex. 2010. Ministry of Food and Drug safety. Republic of Korea.

Lepage, G. and C.C. Roy. 1986. Direct transesterification of classes of lipids in a one-step reaction. J. Lipid Res. 27(1): 114-120.

Manning, R. 2001. Fatty acids in pollen: a review of their importance for honeybees. Bee World. 82: 60-75.

Morgano, M.A., M.C.T. Martins, L.C. Rabonato, R.F. Milani, K. Yotsuyanagi and D.B. Rodriguez-Amaya. 2012. A comprehensive investigation of the mineral composition of Brazilian bee pollen: geographic and seasonal variations and contribution to human diet. J. Braz. Chem. Soc. 24(4): 727-736.

Roulston, T.H. and J.H. Cane. 2000. Pollen nutritional content and digestibility for animals. Plant Syst. Evol. 222: 187-209.

Roulston, T.H, J.H. Cane and S.L. Buchmann. 2000. What governs protein content of pollen: pollinator preference, pollen pistil interactions or phylogeny Eco. Monogr. 70: 617-643.

Seeley, T.D. 1985. Food Collection. pp. 83. in Honeybee Ecology A study of adaptation in social life. Princeton University Press, New Jersy.

Singh, S., K. Saini and K.L. Jain. 1999. Quantitative comparison of lipids in some pollens and their phagostimulatory effects in honeybees. J. Apicult. Res. 38: 87-92.

Szczesna, T. 2007. Concentration of selected elements in honeybee collected pollen. J. Apicult. Sci. 51(1): 5-13.

Yang, K., D. Wu, X. Ye, D. Liu, J. Chen and P. Sun. 2013.Characterization of chemical composition of bee pollen in China. J. Agric. Food Chem. 61: 708-718.